The passage of spermatozoa to regional lymph nodes in testicular lymph following vasectomy in rams and boars

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Summary. Testicular lymph and regional lymph nodes consistently contained spermatozoa in rams and boars up to 3 months after vasectomy. Such direct access of spermatozoa to lymph nodes is likely to provide a powerful stimulus to the development of anti-sperm autoimmunity.

Introduction

Large numbers of spermatozoa have been seen in a para-aortic lymph node in a man 1 year after vasectomy (Ball, Naylor & Mitchinson, 1982) and, rarely, in lumbar aortic lymph nodes of vasectomized Balb/c mice (R. Y. Ball, unpublished observation). Such observations suggest one mechanism which might stimulate the production of autoantibodies against spermatozoa, a phenomenon often seen after vasectomy (Faulk & Fox, 1982). Testicular lymphatic cannulations were performed on rams and boars at intervals after vasectomy to determine whether the passage of spermatozoa in testicular lymph (hereafter referred to as 'spermatolymphia') occurs regularly after vasectomy.

Materials and Methods

Four rams of the Clun Forest breed aged 2½–3½ years (mean 2½ years) and of mean weight 83.5 kg (range 77–96.5 kg) and 4 Large White boars aged 5½–7 months and of mean weight 120 kg (range 93–131 kg) were used.

Rams

Unilateral vasectomy was carried out in mid-October. Under fluothane general anaesthesia and using sterile technique, the left ductus deferens was carefully separated from other tissues and divided between ligatures 5 cm from the cauda epididymidis.

Testicular lymphatics were cannulated on both sides but at different times after vasectomy. To match a control sample with each from the vasectomized side, the rams were paired and opposite sides cannulated at about the same time (Table 1). Using a general anaesthetic and under sterile conditions, an incision was made over the inguinal canal and the cremaster muscle split by blunt dissection. The testicular lymph trunks were identified and a heparinized clear vinyl tube of external diameter 1·20 mm (Dural Plastics and Engineering, Dural, New South Wales, Australia) was tied into one of them so that the open end was directed towards the testis. The remaining

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lymphatics were left undisturbed. An intravenous injection of 20,000 units sodium heparin was administered. The wound was closed in layers, care being taken to maintain haemostasis. Testicular lymph was collected for 2–3 h; the animal was then allowed to recover in a harness for restraint. Lymph was thereafter collected chronically into a heparinized plastic bottle stitched to an elasticated bandage lightly applied round the neck of the scrotum. Samples of lymph were taken as 24-h collections for as long as the catheter remained patent (Table 1). When the second catheter ceased flowing, the animal was anaesthetized again and 1 ml 10% Evans blue in saline was injected into the anterior aspect of each testis to facilitate identification of regional lymph nodes. The ram was killed with an overdose of pentobarbitone sodium 30 min later.

<table>
<thead>
<tr>
<th>Table 1. Times of cannulation of testicular lymphatics and duration of lymph flow in rams</th>
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<tr>
<td>Interval between vasectomy and cannulation of lymphatics (days)</td>
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<tr>
<td>Ram</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>N251</td>
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<tr>
<td>P384</td>
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<tr>
<td>P238</td>
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<tr>
<td>P395</td>
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</table>

**Boars**

Unilateral vasectomy was performed in early March. Each animal was sedated with an intramuscular injection of 500 mg ketamine and general anaesthesia was induced with fluothane and oxygen delivered via a face mask and maintained through an endotracheal tube. The left ductus deferens was ligated and divided about 10 cm from the cauda epididymidis under sterile conditions. A liberal application of sulphathiazole powder was made to the subcutaneous tissues before the wound was closed. An intramuscular injection of 200 mg procaine penicillin and 250 mg dihydrostreptomycin sulphate was administered.

Bilateral testicular lymphatic cannulations were performed 1, 2, 4 or 8 weeks after vasectomy under general anaesthesia, induced as above but maintained with intravenous pentobarbitone sodium. The technique of lymphatic cannulation was similar to that used with the rams except that 2 lymphatics on each side were cannulated and most of the remaining lymph trunks were ligated. In addition, cannulae were inserted into a small superficial vein on the anterior aspect of the right testis, into the right spermatic vein and into the left femoral artery in connection with other experiments not related to this study. Samples of lymph were collected for 3 h. Then 1 ml 10% Evans blue in saline was injected into the testis and the animal killed 30 min later by an overdose of pentobarbitone sodium.

**Specimens**

The lymph samples from rams and boars were centrifuged at 1500–2000 g for 10 min. The pellet was resuspended in about 1/10 volume of saline (9 g NaCl/l) to concentrate the cells. Cell counts were determined using an improved Neubauer chamber and smears were fixed in methanol and stained with haematoxylin and eosin for differential counts.

Necropsy was performed immediately after the animals had been killed. The major testicular axes were measured and wedges of testicular tissue and the lymph nodes draining the testis and nodes from other regions of the body were fixed in Bouin’s solution. The rest of the testes and the attached epididymides were fixed whole in 10% formal saline for at least 1 week. Transverse sections were then made along the entire length of the epididymides at intervals of 2–4 mm and
representative samples were taken from all regions for histological examination. The testes were transected at 1 cm intervals and inspected. Blocks for histological examination were processed using standard techniques and paraffin-wax sections 5 μm thick were stained with haematoxylin and eosin and other special stains as appropriate (e.g. PAS; PAS after diastase digestion; modified Ziehl–Neelsen; Schmorl; Masson–Fontana; oil-red-O; Sudan black; Perls' stain; elastic Ponceau S; and periodic acid–silver).

Results

Rams

Testicular lymph. The mean rate of lymph flow varied from ram to ram and from day to day but was generally in the range 5–10 ml/h, as previously reported by Cowie, Lascelles & Wallace (1964). For 1 or 2 days after cannulation the lymph usually contained a few erythrocytes. The total nucleated cell count varied from undetectable to about 400/μl. Small lymphocytes predominated but other mononuclear cells, presumed to be macrophages and large lymphocytes, formed an important proportion, as did polymorphonuclear neutrophil leukocytes in the first few days. These results are consistent with the findings of Smith, McIntosh & Morris (1970). Lymph samples from the vasectomized side in all rams except No. P238 contained spermatozoa, occasionally in numbers up to 30/μl. They were usually free and often retained their tails; rare sperm heads appeared within macrophages, presumably as a result of phagocytosis. The presence of recognizable spermatozoa was sporadic and there were occasions when none was seen (Text-fig. 1). Ram P395 did not show spermatolymphia until 13 days after lymphatic cannulation. Immediately before this its retaining harness collapsed and this probably caused mild scrotal trauma. Spermatozoa were present in the

![Text-fig. 1. Mean daily numbers of spermatozoa in testicular lymph after cannulation of one of the left testicular lymphatic vessels in a ram. The cannula was inserted 15 days after left unilateral vasectomy and the animal was killed 35 days later. The asterisks indicate days when the samples were not available. Samples after Day 32 did not contain spermatozoa.](https://example.com/text-fig1.png)
testicular lymph for the next 3 days. None of the samples of lymph from the right (control) side contained spermatozoa but they were otherwise similar in all respects to the lymph from the vasectomized side.

Macroscopic findings. The right testes, epididymides and deferent ducts were unremarkable. The left testis in 2 of the rams appeared normal but in the other 2 it was smaller and softer than usual. Both these rams also had large spermatic granulomata in the cauda epididymidis (Table 2). The left efferent ducts and all of the epididymal duct except the cauda showed no macroscopic abnormality. The spermatic granuloma in the cauda of the 2 rams with atrophic testes were approximately spherical and were 1–3 cm in diameter. They consisted of a large central mass of soft semi-liquid sulphur-yellow material surrounded by a thin rim, 1–3 mm thick, of grey-brown tissue. Outside this was a thicker zone of fibrosis. The cauda epididymidis of one of the other rams (P395) was considerably distended by retained cream-coloured fluid but was otherwise unremarkable. The convoluted and straight deferent ducts proximal to the vasectomy site were dilated by similar fluid and appeared fibrotic in places. All rams had 1–4 cm diameter nodules at the vasectomy site. The smaller nodules appeared fibrotic but the large ones (Pl. 1, Fig. 1) were typical spermatic granulomata. All nodules were bound to the parietal tunica vaginalis by fibrous tissue. The rams killed at 34 or 51 days after vasectomy had small amounts of soft creamy material resembling inspissated seminal fluid within the left tunical sac.

Table 2. Ratio of calculated testicular volumes, calculated as $V = \frac{4}{3}\pi abc$ (where $a$, $b$, $c$, are the main perpendicular radii), and the presence of caudal spermatic granulomata in vasectomized rams

<table>
<thead>
<tr>
<th>Ram</th>
<th>Interval between vasectomy and necropsy (days)</th>
<th>Ratio of calculated testicular volumes (L:R)</th>
<th>Spermatic granuloma in cauda epididymidis</th>
</tr>
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<tbody>
<tr>
<td>P384</td>
<td>34</td>
<td>96%</td>
<td>0</td>
</tr>
<tr>
<td>N251</td>
<td>51</td>
<td>72%</td>
<td>+</td>
</tr>
<tr>
<td>P238</td>
<td>83</td>
<td>42%</td>
<td>+</td>
</tr>
<tr>
<td>P395</td>
<td>103</td>
<td>97%</td>
<td>0</td>
</tr>
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</table>

PLATE 1

Fig. 1. Transverse section through left testis (t) of Ram P395 103 days after vasectomy. The corpus epididymidis is indicated by an arrow. A large spermatic granuloma (sg) is related to the site of vasectomy. The soft central mass of spermatozoa and debris is surrounded by a thin rim of grey inflammatory exude, outside which is a thick zone of white, fibrous tissue. Gross specimen, × 0.8.

Fig. 2. Testicular atrophy in a ram 83 days after vasectomy. There appears to be a degree of thickening of the membrana propria of the seminiferous tubules. H & E, × 200.

Fig. 3. Smear of concentrated testicular lymph 8 weeks after vasectomy in a boar. Numerous spermatozoa are present. The remaining cells are lymphocytes and a few erythrocytes. H & E, × 780.

Fig. 4. Large numbers of spermatozoa are present in lymphatic vessels adjacent to a spermatic granuloma of the cauda epididymidis in a boar 2 months after vasectomy. H & E, × 200.

PLATE 2

Fig. 5. Para-aortic lymph node from a boar 8 weeks after unilateral vasectomy. The sinus contains masses of agglutinated spermatozoa which have stimulated a granulomatous reaction. Several multinucleate giant cells are present. H & E, × 260.

Fig. 6. Para-aortic lymph node from a boar 8 weeks after unilateral vasectomy. Large, multinucleate sinus macrophages contain sperm heads and coarse clumps of pigment (arrows). H & E, × 1040.
Lymph nodes draining the testes were easily recognized by their blue colour. They were situated close to the inferior vena cava and abdominal aorta just above the bifurcations of the vessels. Their number ranged from 1 to several on each side.

**Histological findings.** The testes, efferent ducts, epididymides and deferent ducts from the right (control) side showed no histological abnormality. The two left testes of normal size were unremarkable but those that were atrophic showed pathological changes, the severity of which correlated with the apparent loss of volume. Occasional spermatic tubules showed normal spermatogenesis but many others, usually occurring in groups, were considerably shrunken and contained only Sertoli cells and rare spermatogonia (Pl. 1, Fig. 2). Some of the Sertoli cells contained granules of brown, PAS-positive pigment. The membrana propria of such tubules appeared to be thickened with a coarsening of the basement membrane and, possibly, an increased amount of fibro-elastic tissue. Other tubules lay between these extremes and there was apparently production of some cells to the primary spermatocyte stage but not beyond. As well as these changes, the more severely affected testis contained a few interstitial foci of mononuclear inflammatory cells, predominantly lymphocytes. Such lesions usually bore no particular relationship to damaged testicular tubules but one such focus was adjacent to a necrotic tubule invaded by similar mononuclear cells.

Only one ram (No. P238) showed any microscopic abnormalities of the left efferent ducts and of the initial and middle segments of the epididymal duct. These included a few pigmented macrophages, containing phagocytosed spermatozoa, in some of the efferent ducts. The epididymal duct was depleted of spermatozoa and showed thickening of the muscle coat.

The cauda epididymidis was distended by spermatozoa in all rams. The spermatic granulomata which were present in 2 animals showed conspicuous spermiophagy by macrophages. Many such cells contained granules of brown pigment or exhibited basophilic cytoplasm.

The proximal ductus deferens was distended with spermatozoa and had a thickened, fibrotic wall. In some places the epithelium was deficient and replaced by a palisade of macrophages, epithelioid cells and giant cells. The vasectomy site showed a typical foreign-body reaction to the ligature and in Rams N251 and P395 spermatic granulomata were also found there; Ram N251 also showed a granuloma in the cauda epididymidis but Ram P395 did not. In the other 2 animals there were masses of necrotic debris with an associated inflammatory response. Neither lesion contained recognizable spermatozoa. The ductus deferens distant from the testes contained a few spermatozoa in Ram P238 but was empty in the other 3 animals. There was no evidence of re-canalization of the vas or of the characteristic changes of vasis nodosa (Civantos, Lubin & Rywlin, 1972).

The first two rams to be killed showed histological evidence of the escape before death of spermatozoa from the vasectomy site into the tunical sac.

All lymph nodes examined, from the lumbar aortic chain draining the testis, from the pelvis or from the femoral region, contained numerous germinal centres of active appearance. A few large cells containing brown, PAS-positive pigment were present in and around the follicles. The medullary cords contained many plasma cells. Some of the nodes were haemal with free erythrocytes and erythrophagocytosis. Eosinophils were present in large numbers in the nodal pulp. In addition, the nodes draining the testes, in the right and left lumbar aortic chains, showed numerous large paracortical macrophages full of coarse brown pigment granules. The cells occurred singly or in small groups and their pigment was PAS-positive, acid-fast, and stained with Schmorl, oil-red-O and Sudan black, even in paraffin-wax sections. Other macrophages contained large droplets of pale basophilic, strongly PAS-positive material of obscure nature. Lumbar aortic lymph nodes from Ram N251 showed small focal accumulations of large, epithelioid-like cells in the sinuses. Some such cells contained phagocytosed sperm heads. Spermatozoa were also seen in the lymph nodes of Ram P384.

**Boars**

**Testicular lymph.** The rate of lymph flow was considerably greater than in the rams, about 1–2 ml/min, as previously reported by Setchell (1982) and Setchell, Laurie, Flint & Heap (1983). There
were up to 100 nucleated cells/µl, the great majority being small lymphocytes. All samples contained some erythrocytes. Spermatozoa were present in the left testicular lymph from animals vasectomized 1, 2 or 8 weeks previously (Pl. 1, Fig. 3) and in the control lymph 1 week after vasectomy. The spermatozoa had a normal morphology and, in the animal killed at 8 weeks after vasectomy, they were present in large numbers (up to 30/µl). Very rarely a macrophage appeared to contain phagocytosed sperm heads.

**Macroscopic findings.** The right testes, epididymides and deferent ducts presented no significant abnormality. The left testes were normal in size and consistency and the heads and bodies of the epididymides were unremarkable. Animals killed at 1, 2 or 4 weeks showed progressive distension of the cauda epididymidis and ductus deferens by cream-coloured fluid. The boar examined 8 weeks after vasectomy had several spermatic granulomata in the cauda epididymidis. One had ruptured into the tunical sac. All animals showed fibrous nodules, 5–10 mm in diameter, at the vasectomy site. They contained tiny spermatic granulomata. The testicular lymph nodes were similar in number and situation to those of the rams.

**Histological findings.** The right testes and epididymides showed no histological abnormality. The left testes, efferent ducts and initial and middle segments of the epididymides were unremarkable histologically. The cauda epididymidis of boars killed 1, 2 or 4 weeks after vasectomy was distended by retained spermatozoa. The boar killed at 8 weeks showed several large caudal spermatic granulomata with peripheral granulation tissue, fibrosis and focal, mild lymphocytic infiltration. Frozen sections of one spermatic granuloma showed that many of the inflammatory cells contained lipid which stained with oil-red-O. Some, but by no means all, of this was removed by treatment with alcohol and xylene. Nearby lymphatic vessels contained innumerable spermatozoa (Pl. 1, Fig. 4) and some associated intraluminal macrophages. The vasectomy site in all animals showed a foreign-body reaction to the ligature and small spermatic granulomata. Adjacent lymphatic vessels in 2 animals contained spermatozoa. In the animal killed at 8 weeks there was a large focal accumulation of lymphocytes containing numerous germinal centres. There was histological evidence in 3 boars of escape of spermatozoa into the tunical sac before death.

Para-aortic lymph nodes from all animals contained large numbers of sperm heads. They appeared normal histologically and lay in the sinuses, where many had been phagocytosed by macrophages or large epithelioid-like cells. Many phagocytes contained several sperm heads each. In the boar killed at 8 weeks some of the sinuses were dilated and contained large masses of agglutinated spermatozoa surrounded by a prominent granulomatous reaction with macrophages, epithelioid cells and Langhans-type giant cells (Pl. 2, Fig. 5). Spermiophagy was widespread in such lesions but pigmentation was absent. Para-aortic lymph nodes from all boars contained clusters of pigmented cells, similar to those seen in the rams, in the paracortex or lining the sinuses. A few such cells contained phagocytosed sperm heads (Pl. 2, Fig. 6). Germinal centres were large and appeared active and cells containing brown, PAS-positive, weakly acid-fast pigment were seen at their periphery and within their substance. Plasma cells were not conspicuous. Lymph nodes from other regions (e.g. the right spermatic cord (1 animal); near the left renal artery; and from the axilla) appeared active with large germinal centres but contained no spermatozoa and hardly any pigmented cells.

**Discussion**

Testicular lymph, which would also receive a component derived from the epididymis (McBrien, Edwards & Kinmonth, 1972), contained spermatozoa in all but one of the rams and boars after vasectomy. Spermatozoa appeared intermittently from as early as 1 week after operation and as late as 3 months afterwards. Spermatolymphia was seen in only one of the control catheters (and then only occasional spermatozoa were seen) and this probably was the result of minor trauma sustained during the insertion of the testicular venous catheters. Cross-over of lymphatics at or below the level of the inguinal canals is most unlikely.
It seems that spermatozoa enter the lymphatics during the development of interstitial spermatid granulomata. Spermatozoa were recognized in lymph vessels adjacent to such lesions in these experiments and a similar observation has been made with human specimens (Friedman & Garske, 1949; King, 1955; Glassy & Mostofi, 1956). It is probable that spermatozoa reach the lymph soon after they spill into the interstitium and periodically after the inflammatory reaction has become established by breaching its wall of cellular exudate. This may occur as a result of increasing pressure in the fluid centre of the granuloma or of minor trauma (see Ram P395). The observations on smears of lymph cells suggest that most spermatozoa in the lymph are free. However, it is possible that sperm antigens are also transported in the lymph to the regional lymph nodes by the macrophages after phagocytosis and degradation of spermatozoa. Such phagocytosis and degradation occur rapidly in vitro (Ball & Mitchinson, 1977).

Once they reach the regional lymph nodes the spermatozoa are rapidly phagocytosed by sinus macrophages. The latter tend to develop an epithelioid appearance and giant cells are also seen. In the pig examined 8 weeks after vasectomy spermatid granulomata were present within the lymph node sinuses. The spermatozoa appear to be degraded with the production of a ceroid pigment (Pearce, 1972) and the presence of small clusters of macrophages containing such material in the paracortex implies migration of sinus macrophages into the nodal pulp. Such a migration may be important in the development of the immune response (Hoefsmit, Kamperdijk & Balfour, 1980). It is not clear whether the pigment seen in and around the germinal centres is necessarily related to that seen in the sinuses and paracortex. Kotani et al. (1977) noted such cells, which they termed 'Type II phagocytes', in lymph nodes after peripheral injections of India ink and suggested they were derived from afferent lymph.

The lumbar aortic lymph nodes draining the testis also drain other areas of the hind part of the body and the pigment they contained may represent indissoluble remnants derived from a variety of sources. Mitchinson (1982) has pointed out that mononuclear cells containing a ceroid-like insoluble lipid are seen in atherosclerotic arteries and sometimes in neighbouring lymph nodes in humans and has suggested that there may be migration from the former site to the latter. The pigmented macrophages seen in lumbar aortic lymph nodes in the animals studied in the present experiments were probably not derived from such a source because the aortae were judged by macroscopic inspection to be free from atherosclerosis. Moreover, the presence of sperm heads in a few pigmented cells implies a relationship between the degradation of spermatozoa and accumulation of ceroid pigment. Indeed, such a sequence is seen after spermiphagia in vitro (Ball & Mitchinson, 1977).

Spermatolymphia after vasectomy has also been noted in a man 1 year after vasectomy (Ball et al., 1982) and in mice (R. Y. Ball, unpublished observation). The direct access of sperm antigens to regional lymph nodes during the first few weeks after vasectomy correlates extremely well with the time-course of development of serum antisperm antibodies (Faulk & Fox, 1982). It seems likely that spermatolymphia may be a powerful stimulus to the development of such autoantibodies after vasectomy and, presumably, also in some cases of obstructive azoospermia (Rümke & Hellinga, 1959).

Several other abnormalities developed after unilateral vasectomy in our experiments. These included: testicular atrophy in 2 rams; distension of the cauda epididymis and proximal ductus deferens by retained spermatozoa; spermatid granulomata at the vasectomy site and, in a few animals, in the cauda epididymis; and escape of spermatozoa into the tunical sac. None of the boars showed any testicular abnormality up to 8 weeks after vasectomy and spermatogenesis was unaffected in the pigs and in the 2 rams with normal-sized testes. In the rams with atrophic testes after vasectomy, spermatogenesis was disturbed and there were focal inflammatory lesions. These 2 rams also had interstitial spermatid granulomata in the cauda epididymidis, suggesting raised intratubular pressure, at least in the distal epididymal duct. If such increased pressure were transmitted to the seminiferous tubules atrophic changes might occur (Vare & Bansal, 1974). Moore & Oslund (1923–24) noted degenerate testicular tubules in 2 rams vasectomized 76 and 90
days earlier and attributed this to pressure atrophy. Other observations on rams with occluded deferent ducts (but not due to vasectomy) showed that clinical testicular atrophy developed after about 6 months (Tischner, 1971). The early development of testicular atrophy in 2 of the rams in this study may be attributed to the proximal siting of the vasectomy, allowing fewer spermatozoa to be accommodated before pressure built up to a critical level. There was no evidence for other causes of unilateral testicular atrophy such as cryptorchidism or vascular damage. Presumably in the other rams and the 4 boars intraluminal pressure did not reach levels sufficiently high to cause testicular damage. There was no indication that the testes atrophied and then recovered.

Spermatid granulomata developed at the site of vasectomy, in the tunical sac and, in a few animals, in the cauda epididymidis. Their histological appearances were similar to those occurring spontaneously in domestic animals (Jubb, Kennedy & McEntee, 1970). Spermiophagy is one of the most conspicuous features of such lesions and this may be an important mechanism in the development of autoimmune reactions to spermatozoa. However, in men there does not appear to be a correlation between the presence of clinically detectable spermatid granulomata and circulating anti-sperm antibodies so other factors, such as a genetically determined immune response, are probably important too (Alexander, 1977).

The parts of the epididymis which appear to be important for sperm maturation (Glover & Nicander, 1971) showed no significant abnormality after vasectomy except in one ram in which a few luminal spermiophagous cells were noted in the efferent ducts. Similar cells are seen after vasectomy in rhesus monkeys (Alexander, 1972; Tung & Alexander, 1980) and rabbits (Alexander & Tung, 1977) and in men with obstructive azoospermia (Phadke, 1964) and are also observed in so-called ‘brown patches’ of the epididymis (Mitchinson, Sherman & Stainer-Smith, 1975). In rams and boars intraluminal phagocytes does not appear to be an important means of disposing of spermatozoa after vasectomy, at least in the first 2–3 months.

The production of antisperm antibodies after vasectomy may be a consequence of spermiophagy or of lysis of trapped spermatozoa, thereby releasing soluble antigens. However, spermatolymphoma may be a potent stimulus to the development of such autoimmunity and could be at least as important as the other mechanisms.

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References


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